Molecular basis of virus resistance mediated by host factors required for the infectious cycle

Carole Caranta, S. Lacombe, Sophie Ewert, Jean-Luc J.-L. Gallois, Laurence Ouibrahim, André Moretti, Joan Estevan, L. Albar, A. Ghesquiere, J. Bouniol, et al.

2012
PLANT GENOMICS SEMINAR

GIS bV

AGENCE NATIONALE DE LA RECHERCHE
ANR

PONT-ROYAL EN PROVENCE 3-5 APRIL 2012

GENOPLANTE

GIS bV

P44

ANR n°: ANR-08-GEMN-128
Acronym: MOVIe

Total Cost: 2 248 575 euros
Total Grant: 454 082 euros

Date of beginning: 01.01.2009 – **End:** 31.12.2011

ANR

Edition 2008

Molecular basis of virus resistance mediated by host factors required for the infectious cycle

Caranta, C. [1], Lacombe, S.[1], Ewert, S. [1], Gallois, J.L.[1], Ouibrahim, L.[1], Moretti, A.[1], Estevan, J. [1], Albar, L. [2], Ghesquière, A.[2], Bouniol, J.[2], Thiemele, D.[2], Hébrard, E.[3], Fargette, D.[3], Vignols, F.[3], Poulicard, N. [3], Moumene, A.[4], Prado, E.[4], Beuve, M. [4], Dumas, V.[4], Komar, V.[4], Vigne, E.[4], Demangeat, G.[4], Lemaire, O.[4], Merdinoglu, D.[4]

[1] – Partner 1. INRA-UR1052, Genetics and Breeding of Fruits and Vegetable, Dom. St Maurice, BP94, F-84143 Montfavet cedex- [2] - Partner 2. IRD, UMR Génome et Développement des Plantes, av. Agropolis, BP64501, F-34394 Montpellier- [3] - Partner 3. IRD, UMR Résistance des Plantes aux Bioagresseurs, av. Agropolis, BP64501, F-34394 Montpellier- [4] – Partner 4. INRA, UMR Santé de la Vigne et Qualité du Vin, 28 rue de Herrlisheim, BP20507, F-68021 Colmar

Aims

In recent year, one of the most interesting results that has enabled plant virology to make a significant step forward is the identification of components of the translation initiation complex as essential host factors required for RNA virus multiplication. Although translation initiation factors were demonstrated to be highly conserved determinants of plant resistance to viruses, preliminary data indicate that the molecular basis underlying translation initiation factors-mediated resistance are highly variable. In parallel, several recessive resistance genes against viruses were identified and demonstrated to be distinct from translation initiation factors. These genes are therefore very good candidates for the discovery of new susceptibility factors. In this context, the MOVIe project aims at (i) the characterization of mechanisms underlying molecular specificity of translation initiation factors-mediated resistance, (ii) the study of the potential role of translation initiation factors in RNA virus resistance in economically important crops such as grapevine, and (iii) the identification of new host factors required for viral infection The project is likely to provide fundamental insights into the molecular basis of plant-virus interactions and to greatly facilitate the exploitation of host factors required for the viral cycle as targets to improve plant resistance to viruses.

Results (focus on major results)

Broad spectrum resistance to potyviruses in Capsicum results from the combination of AA changes in two regions of the pvr2-eIF4E protein (WP1) - To map *Capcicum* eIF4E key domains and amino acids involved in the interaction with different potyvirus, the diversity analysis of the pepper pvr2- eIF4E locus was extended to 100 *Capsicum* accessions. 14 new allelic variants displaying new combinations of substitutions or new substitutions were identified, including one new allele ($pvr2^{14}$) with broad spectrum resistance to potyviruses. To determine the impact of the different AA changes on the resistance spectrum, 30 eIF4E mutants with one, two or three AA changes were obtained by site-directed mutagenesis and subjected to interaction assays with the VPg of different potyviruses. This assays showed that the combination of two AA changes in two regions of the eIF4E protein is necessary and sufficient to disrupt interaction with all the VPgs.

Common and distinct features of the pathosystem RYMV/rice compared to potyviruses/*Capsicum* (WP1) - The natural polymorphism of the eIF(iso)4G-rymv1 locus is very low compared to eIF4E-pvr2. A strong and direct interaction between the RYMV VPg and the central domain of rice eIF(iso)4G1 was demonstrated by yeast double hybrid assays and in vitro co-purification experiments (Hebrard et al 2010). The mutations of the resistance allele rymv1-2 (and rymv1-5) highly decreased the interaction and the virulence mutations in the VPg restored the interaction. The mutations of the resistance alleles rymv1-3 and rymv1-4 have lower effects. Like PVY in pvr2-resistant pepper, virulence of RYMV in rymv1-resistant rice dependent on epistatic interactions between nearby codons of the VPg. Although the VPgs of potyvirus and RYMV have not the same target (eIF(iso)4G or eIF4E), the global mechanism of resistance and resistance overcoming is similar. However, the two pathosystems have a major distinct feature: the evolutionary scenario for RYMV/rice is different from co-evolution (Hebrard et al 2012). For RYMV, virulence codons of the VPg of wild type isolates are not variable, possibly because resistant cultivars have not yet been deployed widely in the fields, and most importantly, codon 49 – which is a under strong diversifying selection - is not a virulence codon itself but is adjacent to virulence codons 48 and 52.

eIF4E and eIF4G Related sequences as candidates for Nepovirus resistance in grapevine (WP2) - From 12X whole-genome sequencing of the 40024 grapevine accession, we identified a single gene located on chromosome

10 encoding for eIF4E, one gene for isoform eIFiso4E on chromosome 5, one gene for eIF4G on chromosome 15 and two genes for isoform eIFiso4G on chromosomes 4 and 11, respectively. Using a collection of 156 *Vitis vinifera* cultivars and related species, SNP and InDel polymorphisms were identified for eIF4E, eIFiso4E, eIF4G and eIFiso4G in domains putatively involved in the interaction with viral proteins. In parallel, the self-crossing of the studied accessions allowed us to produce S1 progeny for 107 of them. Twenty one of these S1 populations where sequenced for six domains including 16 non-synonymous SNPs. One hundred and sixty five S1 genotypes, homozygous for at least one SNP, were chosen and then planted in a contaminated soil to assess their resistance level to Grape Fanleaf Disease. In order to determine if direct interaction(s) between translation initiation factors and the VPg-Pro domain of GFLV occur, eIF4E, eIFiso4E and eIFiso4G located on chromosomes 10, 5 and 4 respectively, and viral VPg, Pro and VPg-Pro of GFLV-F13 strain have been cloned for Yeast two-hybrid (YTH) experiments. Results indicate that there is no interaction between any *Vitis Vinifera* and *Arabidopsis* translation initiation factors and the viral proteins of GFLV-F13 and ArMV-NW.

A gene involved in constitutive resistance may control both high and partial resistance against RYMV in rice (WP3) - The gene *RYMV2*, conferring high resistance against RYMV in rice, has been located in a 29kb-interval. In parallel, a QTL of partial resistance has been mapped in an 211kb-interval containing the gene *RYMV2*, suggesting that the same gene could be involved both in major and partial resistance. Sequencing of 97% of the 29kb containing *RYMV2* in a highly resistant accession revealed only two polymorphisms compared to accessions with a susceptibility allele. While the first polymorphism is a SNP in a non-coding region, the second one is a deletion in an exon resulting in a frame shift and a truncated, and probably non-functional, protein. Among 115 rice accessions tested, including a majority of susceptible ones, this mutation is present in only four accessions, all resistant. The candidate gene is homologue to an *A. thaliana* gene involved in senescence and constitutive resistance to pathogens.

rwm1 for recessive resistance against *Watermelon mosaic virus* (Potyvirus) in *Arabidopsis thaliana* encodes for a chloroplastic enzyme (WP3) - The characterization of the resistance phenotype against 5 strains of *Water melon mosaic virus* (WMV, genus *Potyvirus*) of the ecotype Cvi showed that resistance is operating at the cellular level i.e., no viral RNA or protein detected on inoculated leaves. Genetic analysis on F1 and F2 progenies showed that a single recessive gene, named *rwm1*, controls Cvi resistance. In order to map *rwm1*, resistance assays were performed on near isogenic introgression lines (introgressions of Cvi genome into a Ler background). *rwm1* was mapped on the long arm of chromosome 1 to a 2.5 Mb-interval. The fine mapping of *rwm1*, performed on F3 progenies with recombinations within the 2.5 Mb-interval (coll. V. De Croocq, INRA Bordeaux), permitted to localize the resistance gene to an interval of 114 kpb, containing 28 genes. Among these genes, a chloroplastic enzyme, previously demonstrated to be involved in plant-potexvirus interactions, was further characterized. Polymorphism analysis together with functional complementation assays (using VIGS in tobacco) confirmed the involvement of this gene in *Arabidopsis* resistance to WMV.

Perspectives

Results from WP1 and WP3 will be published before the end of 2012. Concerning WP2, and in order to determine the putative association between natural variants of eIF4 genes identified in grapevine varieties and the level of resistance to Grape Fanleaf Disease, S1 plants, homozygous for different non-synonymous SNPs, will be analysed by ELISA for two supplementary years (2012 and 2013) after growing in a contaminated soil.

Publications / Congress

- **Bouniol J., Thiémélé D.,** Chéron S**., Ghesquière A**, **Albar L**.. The resistance gene *RYMV2*, mapped in a 52kb-interval, may control both High and partial résistance against RYMV. **2011**, 13ème Rencontres de Virologie Végétale, Aussois, France 23-27/01.
- **Caranta, C., Ouibrahim, L., Lacombe, S.,** Robaglia, C., **Gallois, J.L. 2010**. Host factors required for plant susceptibility to viruses: targets to improve plant resistance. EMBO Workshop Genomic approaches to interactions between plant viruses, their hosts and their vectors. 12-16 June 2010, Fenestrelle, Italy *(Communication orale invitée).*
- **Caranta, C., Ouibrahim, L., Lacombe, S., Salgues, A., Moretti, A., Gallois, J.L. 2010**. The figth for translation : use of translation initiation factors by plant RNA viruses. AAB Meeting, International Advances in Plant Virology, 5-7 September 2010, Arnhem, Netherland. (*Communication orale invitée*).
- **Hébrard E, Poulicard N,** Gérard C, Traoré O, **Albar L, Fargette D,** Bessin Y, Vignols F. 2010. Direct interaction between the Rice yellow mottle virus VPg and the central domain of the rice eIF(iso)4G1 factor correlates with rice susceptibility and RYMV virulence. *Molecular Plant Microbe Interaction*, 23 (11):1506-1513.
- **Lacombe S., Ewert, S.,** Moretti, A., Fouet, C., **Caranta, C. 2011**. Des régions clé de la protéine pvr2-eIF4E1 du piment gouvernent la résistance au potyvirus et son spectre. 13ème Rencontre de Virologie Végétale d'Aussois, 16-20 Janv 2011, Aussois, France. (*Communication orale*).
- LeGall, O., Aranda, M., and **Caranta, C.** 2011. Plant resistance to viruses mediated by translation initiation factors. In *Recent Advances in Plant Virology, Eds C. Caranta, M. Aranda, M. Tepfer and JJ Lopez-Moya,* Caister Academic Press, pp 177-194.
- **Ouibrahim, L.,** Moretti, A., Salgues, A., Giner-Rubio, A., Lecoq, H., Robaglia, C., **Caranta, C. 2011.** Towards the identification of new host factors required for plant susceptibility to potyviruses: novel routes to resistance. 13ème Rencontre de Virologie Végétale d'Aussois, 16-20 Janv 2011, Aussois, France. (*Communication orale*).
- **Poulicard N,** Pinel-Galzi A, Traoré O, **Albar L,** Vignols F, **Ghesquière A,** Konaté G, **Hébrard E, Fargette E.** Ancient host adaptation modulated the actual resistance-breaking ability of the Rice yellow mottle virus. EMBO workshop Genomic approaches to interactions between plant viruses, their hosts and their vectors , 12-16 juin 2010, Fenestrelle, Italy *(poster)*
- **Poulicard N,** Pinel-Galzi A, Traoré O, Vignols F, **Ghesquière A,** Konaté G, **Hébrard E, Fargette D.** 2012. Historical contingencies modulate the adaptability of Rice yellow mottle virus, *PLoS Pathogens*, 8 (1): e1002482.

Total permanent scientist

P1 : 131 hommes/mois - P2: 104 - P3: 40 - P4: 67

Temporary contracts

- Séverine LACOMBE	Post-doctoral fellow, 30 months, Avignon (P1)	From February 2009 to Sept 2010
- Sophie EWERT	Engineer, 12 months, Avignon (P1)	From Sept 2010 to Sept 2011
- Julie BOUNIOL	Engineer, 18 months, Montpellier (P2)	From Nov 2009 to Dec 2011
- Amal MOUMENE	Engineer, 18 months, Colmar (P4)	From March 2009 to August 2010
Not funded by the MOVIe Project :		
- Laurence OUIBRAHIM	PhD student, Avignon (P1)	From January 2009 to Jan 2012
- Deless THIEMELE	PhD student, Montpellier (P2)	From January to September 2009
- Nils POULICARD	PhD student, Montpellier (P3)	From Dec 2007 to Dec 2010